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Cytomegalovirus Antibody Responses Associated With Increased Risk of Tuberculosis Disease in Ugandan Adults

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Background. Recent evidence highlights human cytomegalovirus (HCMV) and immune activation as risk factors for tuberculosis disease. It is not known whether other herpesviruses are also implicated, nor whether a dose-response relationship exists between tuberculosis risk and herpes coinfection.

Methods. This nested case-control study used stored serum samples from 25 persons with tuberculosis up to 10 years before tuberculosis diagnosis and between 3 and 6 matched controls without tuberculosis from a rural Ugandan cohort. Samples were investigated for Epstein-Barr virus, herpes simplex virus, and HCMV-specific immunoglobulin G (IgG), serum markers of inflammation, and mycobacterial antibody levels.

Results. Humoral response to HCMV, but not Epstein-Barr or herpes simplex virus, was associated with increased risk of active tuberculosis disease up to 10 years before diagnosis. Individuals with medium HCMV IgG were 2.8 times more likely to have tuberculosis ($P = .055$), and those with high HCMV IgG 3.4 times more likely to have tuberculosis ($P = .007$). Mycobacterial antibody levels were not associated with differences in odds of tuberculosis disease. Interferon-induced protein 10 was independently associated with increased odds of tuberculosis (odds ratio, 4.2; $P = .009$).

Conclusions. These data provide evidence of a dose response between magnitude of HCMV IgG with risk of tuberculosis disease. An inflammatory environment, characterized by serum interferon-induced protein 10 and interleukin 1α, is independently associated with increased risk of tuberculosis disease.

Keywords. tuberculosis; HCMV; cytomegalovirus; IP-10; CXCL10; Case-control; Uganda.

Epidemiological studies have identified important risk factors for tuberculosis: human immunodeficiency virus (HIV) [1], diabetes [2], interferon (IFN) γ deficiencies [3], and malnutrition [4]. Despite these findings, the vast majority of individuals who have tuberculosis globally are HIV negative, nondiabetic, and immunocompetent. The reasons why they acquire active tuberculosis disease, thereby propagating transmission of the pathogen, are unknown.

Human cytomegalovirus (HCMV), also known as human herpesvirus 5, is a member of the β-herpesviridae subfamily, is widely distributed in human populations, and is transmitted through person-to-person contact. In many areas of the world with the highest burden of tuberculosis, HCMV infection is nearly ubiquitous and evidence exists for convergent epidemiology of the 2 pathogens [5].

Once infected, HCMV establishes lifelong latency in a variety of cell types, including those infected by *Mycobacterium tuberculosis*. Infection rarely results in serious adverse effects in immunocompetent individuals, but it can cause permanent hearing and neurological damage in neonates [6], severe non-AIDS events in HIV-infected persons [7], and important clinical problems in solid organ transplant recipients [8]. Despite its ubiquitous and mostly benign status as an infectious disease, HCMV infection is highly associated with immune variation [9], T-cell activation [10], immune senescence [11], and memory inflation [12]. The biological basis for increased cytomegalovirus immunoglobulin (Ig) G levels is incompletely understood. The results of some studies suggest that accumulated HCMV burden is correlated with high HCMV IgG titers [13, 14]. Seropositivity has been linked to increased overall mortality rates [15], and the presence of virus in blood is linked to a range of chronic diseases [16].

There is epidemiological evidence of elevated HCMV-specific IgG in persons with tuberculosis [17, 18], and 2 studies found a link between increased latent and active HCMV infection in

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persons with tuberculosis and nontuberculous mycobacterial disease [18, 19]. Given the paucity of information of HCMV-associated tuberculosis risk, this study aims to further investigate the possible role of HCMV in tuberculosis disease, using a case-control study based in a rural Ugandan longitudinal cohort. In addition, inflammatory serum markers, mycobacterial antibodies and levels of IgG against 2 other chronic herpesviruses; Epstein-Barr virus (EBV) and herpes simplex virus (HSV) 1 and 2, were investigated to determine associations with tuberculosis risk in this cohort.

METHODS

Sampling

The General Population Cohort (GPC) is a population-based open cohort in rural Uganda. The GPC was established in 1989 to examine trends in prevalence and incidence of HIV infection and their determinants [20]. The cohort comprises approximately 20 000 people, half of whom are aged <13 years. Data are collected through an annual census and blood samples are stored at −80°C in a biobank, located in Entebbe, Uganda. Testing for HIV was carried out immediately after blood collection in Uganda, as described elsewhere by Asiki et al [20]. Tuberculosis is diagnosed through passive case identification of symptomatic individuals presenting for care at GPC clinics. The current study included 25 individuals from this cohort who had sputum-positive active tuberculosis disease diagnosed between 1999 and 2014, and who had available serum samples. All available stored serum samples were retrieved for these 25 persons, from 10 years before and up to 3 months after tuberculosis diagnosis. Between 1 and 4 stored serum samples were identified and retrieved for each tuberculosis case (51 total samples). Figure 1 shows the timing of samples obtained from relative to the time of tuberculosis diagnosis.

For controls, we selected stored serum samples collected in 2011 from people within the GPC who had no record of tuberculosis disease by the end of the dates included in the current study (2014). Samples were matched on known predictors of HCMV level, age, sex and HIV status at the time samples were obtained. Between 3 and 6 control individuals were matched per tuberculosis case sample (with a maximum of 1 sample per control individual). Because of the ubiquity of HCMV infection within this population [17], HCMV-seronegative samples were excluded (n = 9; all 9 from controls without tuberculosis with a mean age of 37 years [range, 26.9–50.8 years]; 2 of 9 controls were HIV positive) The 307 HCMV-positive samples (256 control and 51 tuberculosis samples, grouped into 51 case-control matched sets), were included in the analyses (Table 1), such that tuberculosis cases with multiple samples could be included in >1 set. The exposure of interest was tuberculosis disease as a binary measure.

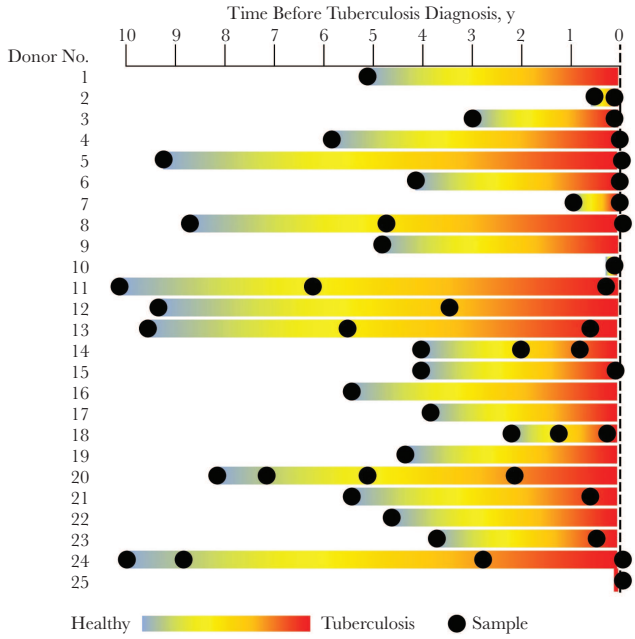

Figure 1. In 25 individuals with diagnosed active tuberculosis disease, samples were obtained before and at the point of tuberculosis diagnosis (1–4 samples per individual; 51 total samples).

Ethical Approval and Consent to Participate

Ethical approval for this study was obtained from London School of Hygiene & Tropical Medicine (references 10000 and 10643), the Uganda Virus Research Institute Research and Ethics Committee (reference GC/127/15/06/512), and from the Uganda Council for Science and Technology. Written consent for the use of clinical records and biological samples for research purposes was obtained from all GPC participants, following Uganda National Council of Science and Technology guidelines.

Herpesvirus-Specific IgG

Measurement of HCMV IgG was conducted as described elsewhere [17]. IgG levels against EBV nuclear antigen 1 and HSV-1 and HSV-2 full antigens were measured using commercial kits (Euroimmun). The resulting measurement (in relative units) was calculated based on a standard curve from the calibration serum samples and based on kit cutoffs.

Serum Cytokines

Luminex multiplex cytokine platform (Merck Millipore) was used to determine the concentrations of IFN-α2, IFN-γ, interleukin 10, 12p40, 12p70, 1Ra, and 6, interleukin 1α (IL-1α), interleukin 1β (IL-1β), IFN-induced protein 10 (IP-10), and tumor necrosis factor α in serum samples. Bio-Plex manager software (version 6.1) was used for bead acquisition and analysis of median fluorescence intensity. These values were converted to picograms per milliliter using the software.

Table 1. Age, Sex, Human Immunodeficiency Virus Status, and Number of Samples for Individuals With Tuberculosis and Controls Without Tuberculosis

	Controls Without Tuberculosis (n = 256)	Individuals With Tuberculosis (n = 25)	Total (n = 281)
Age, mean (range), y	34.2 (2.75–56.5)	36.1 (13.1–56.5)	34.3 (2.75–56.5)
Female sex, no. (%)	157 (61)	15 (60)	172 (61)
HIV infected, no. (%)	59 (23)	8 (32)	67 (24)
Samples per individual, mean no. (range)	1 (1)	2.04 (1–4)	1.09 (1–4)
Samples, total no.	256	51	307

Abbreviation: HIV, human immunodeficiency virus.

Total IgG

Total IgG was measured as described elsewhere [21]. Serum samples were diluted 1:8x10^5 and these, plus IgG standards (134.4–8.4 ng/mL) were incubated on plates coated with mouse anti-human IgG (Abcam ab200699). After washing and incubation with goat anti-human Fc (Abcam ab97225), plates were read and optical density measurements converted into grams per liter by using the standard curve on each plate.

Mycobacterial Antibodies

Antigen 85A (Ag85A) IgG and IgM, lipoarabinomannan (LAM) IgG, purified protein derivative IgG, and combined 6-kDa early secretory antigenic target (ESAT6)–10-kDa culture filtrate protein (CFP10) IgG were measured as described elsewhere [21]. Briefly, 1:100 diluted test serum samples were incubated on plates coated with recombinant Ag85A (Aeras), purified protein derivative (lot 051815KA; Aeras), LAM (NR-14848, BEI Resources), and combined ESAT6/CFP10 (NR-14868 and NR-49425, respectively; BEI Resources) and. After washing and incubation with either goat anti-human IgG–horseradish peroxidase (04-10-20; KPL) or goat anti-human IgM–horseradish peroxidase (Abcam ab97205), absorbance was measured to obtain optical density, a surrogate marker of antibody titer.

Statistical Analyses

Analyses were conducted on all 307 samples to investigate differences between individuals who progressed to diagnosis with active tuberculosis disease with those who remained tuberculosis free. Pearson correlation was used to investigate associations between continuous measurements of HCMV, EBV, and HSV-1/2 IgG levels, as well as IP-10, IL-1α, and herpesvirus IgG levels. HCMV-, EBV-, and HSV-1/2–seropositive samples were categorized into tertiles according to level of specific IgG response. Associations between herpesvirus IgG tertiles, mycobacterial antibodies, inflammatory markers, and tuberculosis disease were investigated using a conditional logistic regression model conditioned on the 51 matched case-control sets. HCMV tertile was included as a covariate. Owing to different measurement ranges, all continuous exposure variable measurements were *z* score transformed; hence, reported odds ratios (ORs) are for a 1–standard deviation (SD) of the mean increase in response. We investigated the slope of serum antibody and cytokine changes in samples from individuals with tuberculosis over time to diagnosis, using a linear random effects model.

To account for multiple comparisons, 99% confidence intervals (CIs) are reported and a *P* value of .01 is considered strong evidence to reject the null hypothesis. A robust standard error was used in conditional regression analysis to account for the fact that some individuals with tuberculosis contributed >1 sample. All analyses were performed using Stata software (version 14; StataCorp).

RESULTS

Of the 281 HCMV-seropositive individuals included in this nested case control study, 24% were HIV positive (8 of 25 individuals with tuberculosis and 59 of 256 controls; Table 1). Of the 307 HCMV-positive samples included in analyses, 81% (250 of 307) were EBV positive, and 98% (302 of 307) were HSV positive. To examine the robustness of the assay indicating HCMV, EBV, and HSV-1/2 seropositivity, longitudinal samples from the tuberculosis cases were investigated. Of the 17 persons with tuberculosis who contributed >1 sample to analyses, seropositivity was consistent between samples taken from the same

Table 2. Conditional Logistic Regression Model: Mycobacterial Antibodies and Odds of Tuberculosis Disease

	Raw OD Readings	Unadjusted Model		HCMV-Adjusted Model	
Mycobacterial Antibody	OD, Mean (SD)	OR (99% CI)	*P* Value	OR (99% CI)	*P* Value
Ag85A IgG	0.83 (0.4)	0.962 (.645–1.436)	.80	0.919 (.624– 1.353)	.57
PPD IgG	1.14 (0.36)	1.035 (.687–1.559)	.83	1.059 (.704–1.592)	.72
LAM IgG	2.00 (0.58)	1.515 (.948–2.424)	.02	1.51 (.925–2.467)	.03
Ag85A IgM	1.27 (0.82)	1.026 (.666–1.579)	.88	0.912 (.590–1.409)	.58
ESAT6/CFP10 IgG	0.79 (0.56)	1.434 (.974– 2.110)	.02	1.35 (.916– 1.982)	.05

Abbreviations: Ag85A, antigen 85A; CFP10, 10-kDa culture filtrate protein; CI, confidence interval; ESAT6, 6-kDa early secretory antigenic target; HCMV, human cytomegalovirus; IgG and IgM, immunoglobulin G and M; LAM, lipoarabinomannan; OD, optical density; OR, odds ratio; PPD, purified protein derivative; SD; standard deviation.

individual, with 1 individual seroconverting to HSV-1/2 positive and 2 seroconverting to EBV positive during the period for which samples were available. HCMV IgG and HSV-1/2 IgG were positively associated (ρ = 0.20; $P < .001$). HCMV IgG levels were not associated with EBV IgG levels (ρ = −0.04; $P = .47$), nor were EBV IgG levels associated with HSV-1/2 IgG levels (ρ = −0.03; $P = .65$).

Mycobacterial Antibody Levels and Risk of Progression to TB

LAM IgG and ESAT6/CFP10 IgG levels (measured at all time points before and at the time of tuberculosis diagnosis) were associated with 1.5 and 1.4 times increased odds of tuberculosis disease respectively per 1-SD increase in antibody level; however, neither of these were significant at the $P < .01$ threshold. The ORs were slightly reduced when HCMV tertile was included in the regression model (Table 2).

HCMV, HSV, EBV and risk of TB

The odds associated with progression to active tuberculosis were increased 2.8 times among individuals with medium HCMV IgG levels compared with low IgG levels (99% CI, .908–8.638; $P = .055$) (Table 3 and Figure 2). HCMV IgG levels in the upper tertile of the range was associated with a 3.4 times greater odds of having active tuberculosis disease compared with low HCMV IgG levels (99% CI, 1.072–11.074; $P = .007$), the directional trend to increased risk of tuberculosis with increased HCMV IgG was significant ($P = .006$). The same trend of increased risk with higher IgG was not seen with either of the herpesviruses, EBV or HSV-1/2 (Table 3).

CXCL10(IP-10) and IL-1a and Odds of TB

IP-10 is associated with 4.6 times increased odds of progression to tuberculosis disease per 1-SD increase in cytokine level (OR, 4.587; 99% CI, 1.064–19.769; $P = .007$). The OR does not decrease significantly when HCMV IgG levels are added into the conditional logistic regression model (OR, 4.233; 99% CI, 1.021–17.547; $P = .009$) (Table 3). The only other cytokine with a statistically significant association with risk of tuberculosis disease is IL-1α; a 1-SD increase in IL-1α is associated with a 1.5 times greater odds of developing tuberculosis disease (OR, 1.521; 99% CI, 1.045–2.212; $P = .004$) (Table 4).

Correlation of CXCL10(IP-10) with HCMV

Correlations between levels of IP-10 and HCMV, EBV and HSV-1/2 IgG showed that serum levels of IP-10 were positively correlated with increased HCMV IgG levels (ρ = 0.40; $P < .001$), whereas no association was seen between IP-10 and EBV IgG (ρ = −0.07; $P = .22$), or between IP-10 and HSV-1/2 IgG (ρ = 0.03; $P = .54$) (Supplementary Figure 1). IL-1α levels were negatively associated with HCMV and HSV-1/2 IgG levels (ρ = −0.16 [$P = .005$] and ρ = −0.17 [$P = .003$], respectively), and IL-1α levels were not significantly associated with EBV IgG levels (ρ = −0.03; $P = .60$) (Supplementary Figure 2).

Serum HCMV IgG Levels, IP-10 and Prediction of Risk of TB Disease

To explore whether the risk of tuberculosis disease associated with HCMV IgG tertile was independent of that seen with IP-10 and IL-1α, these were included in a conditional logistic regression including HCMV tertile. The addition of IP-10 into the regression model resulted in a better fit ($R^2 = 0.29$) than IL-1α ($R^2 = 0.10$) and so was investigated further. The inclusion of IP-10 modified the OR associated with the medium tertile of HCMV IgG from 2.8 to 1.9, and the OR associated with the highest HCMV IgG tertile from 3.4 to 2.4. Meanwhile the inclusion of HCMV in the model modified the OR associated with IP-10 from 4.6 to 4.2 (Table 3). Although HCMV tertile explained some of the increased risk associated with IP-10 and vice versa, a likelihood ratio test provided good evidence that a model including both HCMV IgG tertile and IP-10 independently, resulted in a statistically significant improvement in

Table 3. Odds of Tuberculosis Disease by Chronic Herpesvirus Immunoglobulin G Level[a]

Herpesvirus IgG Level (Range)	Samples, No.	OR (99% CI)	*P* Value for Trend[b]
HCMV			
Low (0.52–1.03 OD)	102	1.0	.006
Medium (1.04–1.34 OD)	102	2.801 (.908–8.638)	
High (1.35–2.84 OD)	103	3.446 (1.072–11.074)	
HSV-1/2			
Low (25–132 RU)	100	1.0	.17
Medium (133–163 RU)	101	0.811 (.280–2.348)	
High (164–245 RU)	101	0.527 (.149–1.862)	
EBV			
Low (22–61 RU)	83	1.0	.48
Medium (62–106 RU)	83	1.429 (.451–4.528)	
High (107–237 RU)	84	0.717 (.208–2.471)	

Abbreviations: CI, confidence interval; EBV, Epstein-Barr virus; HCMV, human cytomegalovirus; HSV, herpes simplex virus; IgG, immunoglobulin G; OD; optical density; OR, odds ratio; RU, relative units.

[a]Medium and high tertiles are compared with the lowest tertile of IgG level for each virus in a conditional logistic regression model.

[b]*P* values from a likelihood ratio test for trend.

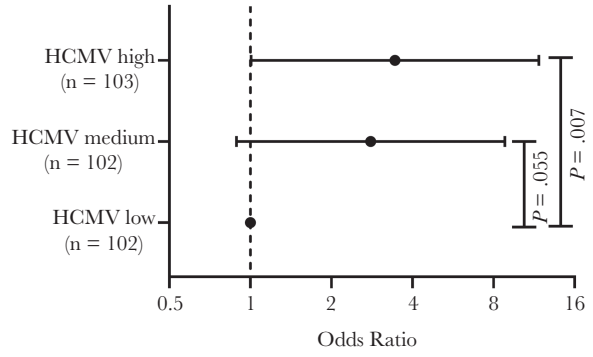

Figure 2. Odds of tuberculosis disease by human cytomegalovirus (HCMV) immunoglobulin G. Results shown are from a conditional logistic regression model, with "HCMV low (0.52–1.03 OD) based on table 3" is used as the reference category. Horizontal lines represent 99% confidence intervals.

model fit ($P < .001$). In conclusion, of the parameters studied here, the best model for prediction of risk of tuberculosis disease includes both HCMV IgG and serum IP-10.

Evidence of Longitudinal Changes Among Individuals With Tuberculosis

There was limited statistical power to detect trends over time; however, among the 17 tuberculosis-positive individuals who contributed >1 sample to analyses, there was evidence that levels of Ag85A IgG, and IL-1α and total levels of IgG changed over time, leading up to the diagnosis of tuberculosis disease (Supplementary Figures 3–5). Both Ag85A IgG (Supplementary Figure 3) and IL-1α (Supplementary Figure 4) showed a trend toward decreased serum levels from 10 years before tuberculosis diagnosis until the point of diagnosis (slope coefficients, −0.04 [99% CI, −0.07 to −.01; $P = .001$] and −14.69 (−28.46 to −.93; $P = .006$], respectively). Total IgG (Supplementary Figure 4) showed a trend toward increasing over time to tuberculosis diagnosis (slope coefficient, 2.10 ;99% CI, .32–3.87; $P = .002$).

DISCUSSION

Using a case-control design containing longitudinal samples obtained up to 10 years before tuberculosis diagnosis, we show that magnitude of HCMV infection, as measured by IgG, is associated with the risk of tuberculosis disease, in a dose-dependent manner. The same association with tuberculosis risk was not seen with EBV or HSV, despite evidence of coprevalence of these 3 chronic herpesviruses [22]. In the current study, we see that risk of tuberculosis disease is increased 3.4 times in those with the highest HCMV levels of IgG. There are a variety of possible mechanisms by which HCMV infection may exacerbate *M. tuberculosis* infection and lead to increased tuberculosis disease risk. HCMV encodes viral proteins that may interfere with protective immune responses, including UL111A, a homologue to the immunosuppressive cytokine interleukin 10 [23]; gpUS6, which blocks peptide presentation, reducing CD8 T-cell recognition [24]; and US2, which causes degradation of HLA-DR-α and DM-α, 2 essential proteins in the major histocompatibility complex class II antigen presentation pathway, thereby blocking CD4 T-cell presentation of viral antigens [25].

Many tuberculosis-endemic areas have very high HCMV seropositivity rates [26, 27]. If HCMV reactivation and reinfection events are driving HCMV IgG in this population, the large number of potential HCMV reactivation and reinfection events possible, given the early age at which an individual is infected with HCMV in this population (83% seropositivity by 1 year of age [17]), makes HCMV-tuberculosis an interesting coinfection model in which to further study mechanisms. Owing to lack of cellular material in this study, cellular interactions were not investigated; however, the propensity of both *M. tuberculosis* and HCMV to infect the same cell type [28, 29] may be an indication of a direct interaction between the 2 pathogens. Alternatively, myriad indirect interactions by 1 pathogen altering the immunologic milieu in favor of a more

Table 4. Conditional Logistic Regression Model: Cytokine and Total Immunoglobulin G Levels and Odds of Tuberculosis Disease

		Unadjusted Model		HCMV-Adjusted Model	
Cytokines and Total IgG	Level, Mean (SD), pg/mL	OR (99% CI)	*P* Value	OR (99% CI)	*P* Value
IFN-α2	16.55 (40.03)	1.412 (.894–2.230)	.052	1.403 (.898–2.192)	.050
IFN-γ	3.94 (11.22)	1.238 (.902–1.700)	.08	1.206 (.881–1.651)	.12
IL-10	20.24 (160.02)	1.763 (.699–4.449)	.12	1.893 (.695–5.154)	.10
IL-12p40	5.34 (30.2)	1.033 (.739–1.445)	.80	1.002 (.708–1.418)	.99
IL-12p70	0.93 (3.39)	0.956 (.671–1.362)	.74	0.982 (.699–1.380)	.89
IL-1Ra	11.93 (67.97)	1.168 (.802–1.700)	.29	1.219 (.908–1.637)	.08
IL-1α	51.67 (122.07)	1.415 (.976–2.051)	.02	1.521 (1.045–2.212)	.004
IL-1β	10.63 (51.41)	0.955 (.754–1.209)	.61	1.022 (.820–1.274)	.80
IL-6	117.3 (536.12)	1.101 (.795–1.525)	.45	1.147 (.857–1.536)	.22
IP-10	279.7 (577.57)	4.587 (1.064–19.769)	.007	4.233 (1.021–17.547)	.009
TNF-α	23.02 (85.85)	0.977 (.772–1.236)	.80	1.023 (.788–1.330)	.82
Total IgG	68.78 (16.21)	0.640 (.388–1.055)	.02	0.590 (.342–1.018)	.01

Abbreviations: CI, confidence interval; HCMV, human cytomegalovirus; IFN, interferon; IgG, immunoglobulin G; IL-1α, IL-1β, IL-1Ra, IL-6, IL-10, IL-12p40, and IL-12p70, interleukin 1α, 1β, 1Ra, 6, 10, 12p40, and 12p70; IP-10, IFN-induced protein 10; OR, odds ratio; SD, standard deviation; TNF, tumor necrosis factor.

favorable environment for persistence of the other pathogen may drive copathogenicity.

In the current study, both IP-10 and IL-1α serum levels were associated with increased risk of tuberculosis (4.2 and 1.5 times, respectively). Despite a positive correlation between IP-10 and HCMV IgG levels in serum, these associations were independent of HCMV IgG levels. Assuming independence, a 1-SD increase in IP-10 combined with a HCMV IgG level in the highest tertile is associated with a 10 times increased odds of tuberculosis disease (OR for HCMV [2.4] multiplied by OR for IP-10 [4.2]) compared with a 1-SD increase in IP-10 in the lowest HCMV tertile. Elevated levels of IP-10 have been associated with posttransplantation morbidity rates in HCMV discordant recipients [11]. In addition, IP-10 has been investigated as a possible diagnostic biomarker for tuberculosis disease (reviewed in [30]); however, its potential role in exacerbating an inflammatory response, leading to a more tuberculosis-permissive environment and tuberculosis disease progression, has not been previously explored to our knowledge.

Emerging evidence suggests that HCMV may induce host production of IP-10. Initially assumed to be a silent infection, the life-long latency established after initial infection with HCMV is characterized by low-level viral gene expression and induction of cytokine production from infected cells [29]. In an experimental latent HCMV model of infected monocytes, selective expression of proinflammatory cytokines, including IP-10, was seen up to 6 days after infection [31]. Although it is likely that there are other causes of elevated IP-10 levels not investigated here, our findings indicate that the risk of active tuberculosis disease associated with HCMV infection might be greater than that attributed to HCMV IgG levels alone. Further investigation is needed to understand the causal link between HCMV and IP-10, but the combined risk of cumulative HCMV infection (as measured by HCMV IgG), in addition to HCMV-induced IP-10–associated risk, may demonstrate that HCMV is acting through multiple mechanisms to increase the risk of tuberculosis. HCMV load was shown to be a poor determinant of mortality rate in very ill HIV- and tuberculosis-coinfected individuals [32], indicating that HCMV IgG may be a better marker for the risk of tuberculosis disease, likely being a result of cumulative reactivation as well as reinfection events throughout a lifetime.

Interleukin 1 has been identified as critical in control of tuberculosis infection [33]. IL-1α and IL-1β dysregulation by type I IFNs (IFN-α and IFN-β) has been linked to disease exacerbation via eicosanoid imbalance–induced necrotic, as opposed to apoptotic, cell death and subsequent bacterial escape and further cellular infection [34]. Although in the current study we see a link between increased IL-1α and increased risk of tuberculosis, rather than interleukin 1 being associated with control of tuberculosis disease, we do have limited evidence to suggest that levels of IL-1α may be decreasing as individuals progress toward diagnosed active tuberculosis disease. We also do not see a concomitant increase or decrease in IL-1β or IFN-α levels; however, many samples were below the lower limit of quantitation for the assay.

In contrast to the protective effect of Ag85A, as seen among BCG-vaccinated South African infants [35], we do not see a difference in serum Ag85A IgG levels between individuals with tuberculosis and matched controls. Despite not finding evidence of a difference in antibodies against 5 mycobacterial antigens between these 2 groups, we did see a trend toward decreased Ag85A IgG levels with time to tuberculosis diagnosis over the preceding 10-year period, which may merit further investigation. In the same population, previous work found an increase in mycobacterial antibody levels with age regardless of tuberculosis status until approximately 20 years of age, suggestive of exposure to nontuberculous mycobacteria, and to *M. tuberculosis* itself [21].

The tuberculosis field has seen a resurgent interest in antibodies in tuberculosis, despite the failure to identify a satisfactory antibody profile that could function as a diagnostic for tuberculosis [36]. A group in China found that total immunoglobulin purified from 7 of 48 tuberculosis-exposed but healthy healthcare workers protected mice against virulent tuberculosis challenge, whereas immunoglobulin from patients with tuberculosis did not protect challenged mice [37]. Using a systems serology approach, Lu et al [38] found a functional role for antibodies in tuberculosis, whereby antibodies from latently infected persons (indicative of control) had enhanced ability to induce phagolysosomal fusion and inflammasome activation, compared with those from persons with active disease.

In summary, the current work shows, for the first time, that the magnitude of humoral responses against HCMV are associated with risk of tuberculosis disease. In addition, an inflammatory environment, possibly exacerbated by HCMV infection itself, is also associated with increased risk of tuberculosis disease in this cohort. Given the ubiquity of HCMV exposure in tuberculosis-endemic settings, and the excess tuberculosis disease risk associated with increased HCMV IgG responses seen in our study, further research should be conducted to determine whether repeated HCMV reinfection and reactivation events are driving this effect and whether HCMV-specific interventions could be investigated as a way to reduce risk of progression to tuberculosis disease. Development of a HCMV vaccine is already underway [39], but target groups include mainly women of childbearing age, to protect neonates from HCMV-associated neurological disorders. The data presented herein show that the need for HCMV control measures may be greater than initially considered, because controlling HCMV could contribute to the control of tuberculosis disease.

The current study had some limitations. Although the GPC has the capacity to collect peripheral blood mononuclear cells, cells were not available for the current study. Control samples were

matched to case samples for age, sex, and HIV status when the sample was obtained, as opposed to matching at point of diagnosis which may introduce a potential source of bias. Our study was also limited by the small number of tuberculosis cases. Reliance on passive tuberculosis case detection in the GPC meant that controls included in this study were not investigated for tuberculosis infection. More stringent inclusion criteria for controls may have resulted in larger differences between groups. As another limitation, the grouping into tertiles of HCMV-specific IgG is based on the ranges seen in our population and may not be generalizable. It will be important to measure ranges in other populations and determine whether specific cutoffs of HCMV IgG levels are associated with increased risk of tuberculosis. Although we had longitudinal data for some individuals with diagnosed tuberculosis, for controls without tuberculosis we had data from only a single time point. Ideally, we would have included longitudinal data for controls, but those samples were not available.

Supplementary Data

Supplementary materials are available at The *Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Author contributions. L. S. conducted laboratory assays, analyzed data, and wrote the manuscript. S. N., R. N., and H. A. F. designed the study and supervised the work. S. N. and R. F. designed the statistical analyses. J. R. and S. M. contributed to analysis and interpretation of results. All authors read, edited, and approved the final manuscript.

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References

1. Selwyn PA, Hartel D, Lewis VA, et al. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. N Engl J Med **1989**; 320:545–50.
2. Harries AD, Kumar AMV, Satyanarayana S, et al. Addressing diabetes mellitus as part of the strategy for ending TB. Trans R Soc Trop Med Hyg. **2015**; 110:173–9.
3. Dorman SE, Holland SM. Mutation in the signal-transducing chain of the interferon-gamma receptor and susceptibility to mycobacterial infection. J Clin Invest **1998**; 101:2364–9.
4. Cegielski JP, McMurray DN. The relationship between malnutrition and tuberculosis: evidence from studies in human and experimental animal. Int J Tuberc Lung Dis. **2004**; 8:286–98.
5. Cobelens F, Nagelkerke N, Fletcher H. The convergent epidemiology of tuberculosis and human cytomegalovirus infection. F1000Res **2018**; 7:280.
6. Marsico C, Kimberlin DW. Congenital cytomegalovirus infection: advances and challenges in diagnosis, prevention and treatment. Ital J Pediatr **2017**; 43:38.
7. Lichtner M, Cicconi P, Vita S, et al; ICONA Foundation Study. Cytomegalovirus coinfection is associated with an increased risk of severe non-AIDS-defining events in a large cohort of HIV-infected patients. J Infect Dis **2015**; 211:178–86.
8. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis **2002**; 34:1094–7.
9. Brodin P, Jojic V, Gao T, et al. Variation in the human immune system is largely driven by non-heritable influences. Cell **2015**; 160:37–47.
10. Slyker JA, Rowland-Jones SL, Dong T, et al. Acute cytomegalovirus infection is associated with increased frequencies of activated and apoptosis-vulnerable T cells in HIV-1-infected infants. J Virol **2012**; 86:11373–9.
11. Berg PJ van de, Heutinck KM, Raabe R, et al. Human cytomegalovirus induces systemic immune activation characterized by a type 1 cytokine signature. J Infect Dis **2010**; 202:690–9.
12. Chidrawar S, Khan N, Wei W, et al. Cytomegalovirus-seropositivity has a profound influence on the magnitude of major lymphoid subsets within healthy individuals. Clin Exp Immunol **2009**; 155:423–32.
13. Dollard SC, Keyserling H, Radford K, et al. Cytomegalovirus viral and antibody correlates in young children. BMC Res Notes. **2014**; 7:2–5.
14. Mehta SK, Stowe RP, Feiveson AH, Tyring SK, Pierson DL. Reactivation and shedding of cytomegalovirus in astronauts during spaceflight. J Infect Dis **2000**; 182:1761–4.

15. Savva GM, Pachnio A, Kaul B, et al; Medical Research Council Cognitive Function and Ageing Study. Cytomegalovirus infection is associated with increased mortality in the older population. Aging Cell **2013**; 12:381–7.
16. Nikitskaya E, Lebedeva A, Ivanova O, et al. Cytomegalovirus-productive infection is associated with acute coronary syndrome. J Am Heart Assoc. **2016**; 5:1–13.
17. Stockdale L, Nash S, Nalwoga A, et al. Human cytomegalovirus epidemiology and relationship to tuberculosis and cardiovascular disease risk factors in a rural Ugandan cohort. PLoS One **2018**; 13:e0192086.
18. Olaleye OD, Omilabu SA, Baba SS. Cytomegalovirus infection among tuberculosis patients in a chest hospital in Nigeria. Comp Immunol Microbiol Infect Dis **1990**; 13:101–6.
19. Amran FS, Kim K, Lim A, et al. Is pulmonary non-tuberculous mycobacterial disease linked with a high burden of latent cytomegalovirus? J Clin Immunol **2016**; 36:113–6.
20. Asiki G, Murphy G, Nakiyingi-Miiro J, et al; GPC team. The General Population Cohort in rural south-western Uganda: a platform for communicable and non-communicable disease studies. Int J Epidemiol **2013**; 42:129–41.
21. Stockdale L, Nash S, Nalwoga A, et al. HIV, HCMV and mycobacterial antibody levels: a cross-sectional study in a rural Ugandan cohort. Trop Med Int Heal. **2018**; 00:1–11.
22. Delaney AS, Thomas W, Balfour HH Jr. Coprevalence of Epstein-Barr virus, cytomegalovirus, and herpes simplex virus type-1 antibodies among United States children and factors associated with their acquisition. J Pediatric Infect Dis Soc **2015**; 4:323–9.
23. Avdic S, Cao JZ, Cheung AK, Abendroth A, Slobedman B. Viral interleukin-10 expressed by human cytomegalovirus during the latent phase of infection modulates latently infected myeloid cell differentiation. J Virol **2011**; 85:7465–71.
24. Halenius A, Momburg F, Reinhard H, Bauer D, Lobigs M, Hengel H. Physical and functional interactions of the cytomegalovirus US6 glycoprotein with the transporter associated with antigen processing. J Biol Chem **2006**; 281:5383–90.
25. Tomazin R, Boname J, Hegde NR, et al. Cytomegalovirus US2 destroys two components of the MHC class II pathway, preventing recognition by $CD4^+$ T cells. Nat Med **1999**; 5:1039–43.
26. Bates M, Brantsaeter AB. Human cytomegalovirus (CMV) in Africa: a neglected but important pathogen. J Virus Erad **2016**; 2:136–42.
27. Kothari A, Ramachandran VG, Gupta P, Singh B, Talwar V. Seroprevalence of cytomegalovirus among voluntary blood donors in Delhi, India. J Health Popul Nutr **2002**; 20:348–51.
28. Ganbat D, Seehase S, Richter E, et al. Mycobacteria infect different cell types in the human lung and cause species dependent cellular changes in infected cells. BMC Pulm Med. **2016**; 16:1–16.
29. Goodrum FD, Jordan CT, High K, Shenk T. Human cytomegalovirus gene expression during infection of primary hematopoietic progenitor cells: a model for latency. Proc Natl Acad Sci U S A **2002**; 99:16255–60.
30. Ruhwald M, Aabye MG, Ravn P. IP-10 release assays in the diagnosis of tuberculosis infection: current status and future directions. Expert Rev Mol Diagn **2012**; 12:175–87.
31. Noriega VM, Haye KK, Kraus TA, et al. Human cytomegalovirus modulates monocyte-mediated innate immune responses during short-term experimental latency in vitro. J Virol **2014**; 88:9391–405.
32. Schutz C, Barr D, Andrade BB, et al. Clinical, microbiologic, and immunologic determinants of mortality in hospitalized patients with HIV-associated tuberculosis: a prospective cohort study. PLoS Med **2019**; 16:e1002840.
33. Mayer-Barber KD, Andrade BB, Barber DL, et al. Innate and adaptive interferons suppress IL-1α and IL-1β production by distinct pulmonary myeloid subsets during *Mycobacterium tuberculosis* infection. Immunity **2011**; 35:1023–34.
34. Mayer-Barber KD, Andrade BB, Oland SD, et al. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature **2014**; 511:99–103.
35. Fletcher HA, Snowden MA, Landry B, et al. T-cell activation is an immune correlate of risk in BCG-vaccinated infants from the MVA85A efficacy trial. Nat Commun. **2016**;:1–10.
36. Broger T, Basu Roy R, Filomena A, et al. Diagnostic performance of tuberculosis-specific IgG antibody profiles in patients with presumptive tuberculosis from two continents. Clin Infect Dis **2017**; 64:947–55.
37. Li H, Wang XX, Wang B, et al. Latently and uninfected healthcare workers exposed to TB make protective antibodies against Mycobacterium tuberculosis. Proc Natl Acad Sci U S A **2017**; 114:5023–8.
38. Lu LL, Chung AW, Rosebrock TR, et al. A functional role for antibodies in tuberculosis. Cell **2016**; 167:433–43.
39. Plotkin SA, Boppana SB. Vaccination against the human cytomegalovirus. Vaccine **2018**. doi:10.1016/j.vaccine.2018.02.089